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# Nutritional Modulation of the Inflammatory Bowel Response

Orestis Ioannidis Ioannis Varnalidis George Paraskevas Dimitrios Botsios

Fourth Surgical Department, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece

**Key Words**
Crohn's disease · Oxidative stress · Ulcerative colitis

**Abstract**
Crohn's disease and ulcerative colitis represent distinct phenotypic forms of inflammatory bowel disease and continue to be a common cause of morbidity. The corticosteroids and the immunomodulatory drugs, which are the basis of treatment for the inflammatory bowel diseases, do not assure always satisfactory outcomes. Nutrition has been used in order to modify the inflammatory response of various chronic inflammatory diseases, including Crohn's disease and ulcerative colitis. In the pathogenesis of inflammatory bowel diseases, the intestinal microflora and the intestinal mucosal disorders play a crucial role. Also, the release of reactive oxygen species is a significant factor of initiation and preservation of the inflammatory reaction in these diseases. The advantages of the nutritional treatment derive from the sequestration of intraluminal agents which may promote the inflammatory bowel response or, alternatively, nutrition is able to modify the immune response, reducing the uncontrolled inflammatory reaction. Furthermore, nutrition can enhance the mucosal barrier function and consists a significant source of antioxidants. This review focuses on certain nutritional components that modulate the inflammatory response of the bowel and aims to present a rational thesis regarding the use of nutritional agents in the management of inflammatory bowel diseases.



## Introduction

Inflammatory bowel diseases (IBD) continue to be a common cause of morbidity and to aggravate the quality of life. Despite the intense research efforts and the considerable progress, the understanding of the pathophysiological mechanisms of IBD remains unclear.

Crohn's disease and ulcerative colitis represent distinct phenotypic forms of inflammatory bowel disease. Their pathogenesis is considered to include disorders of the immunomodulation of the bowel mucosa which leads in lesions of the epithelial cells caused by activated T cells, mononuclear cells and macrophages and there are a lot of indications about the crucial role of the intestinal microflora and the intestinal mucosal disorders in the pathogenesis of the IBD.

The corticosteroids and the immunomodulatory drugs, which are the basis of treatment for the IBD, do not

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Dr. O. Ioannidis
Alexandrou Mihailidi 13
GR–54640 Thessaloniki (Greece)
Tel. +30 231 084 5470, Fax +30 231 055 1301
E-Mail telonakos@hotmail.com

**Table 1.** Mechanism of action of nutritional therapeutic treatment

- Sequestration of intraluminal antigens
- Modulation of the immune response of the bowel
- Amelioration of the antioxidant status
- Alteration in the uptake of polyunsaturated fatty acids (n–6/n–3 fatty acids)
- Enhancement of the restoration/function of the intestinal mucosal barrier
- Regulation of the intestinal microflora
- Regulation of the intestinal motility
- Regulation of the bile-pancreatic secretions
- Sequestration of nutritional particles

**Table 2.** Nutritional substances that can modulate inflammatory bowel disease

- Antioxidants
  - Glutathione
  - Vitamins A, C and E
  - Free metals: selenium, copper, zinc
- Omega-3 polyunsaturated fatty acids
- Short-chain fatty acids
- Amino acids
- Prebiotics and probiotics
- N-acetylglucosamine
- Dietary particles

always assure satisfactory outcomes. Nutriceuticals have been used in order to modify the inflammatory response of various chronic inflammatory diseases, including IBD. For at least three decades, various elemental diets have been utilized in order to control the activity of Crohn's disease. The importance of an elemental diet in remission of the disease both in adults and in children is known [1].

The possible mechanisms of action of the nutritional therapeutic treatment for the control of the activity of the IBD are: (a) sequestration of intraluminal antigens, (b) modulation of the immune response of the bowel, (c) amelioration of the antioxidant status, (d) alteration in the uptake of polyunsaturated fatty acids (n–6/n–3 fatty acids), (e) enhancement of the restoration/function of the intestinal mucosal barrier, (f) regulation of the intestinal microflora, (g) regulation of the intestinal motility, (h) regulation of the bile-pancreatic secretions, and (i) sequestration of nutritional particles (table 1). Although meta-analysis has shown a statistically significant advantage of corticosteroids against specific forms of nutritional treatment, regarding the percentage of remission in patients with Crohn's disease [2, 3], there are clinical conditions in which nutrition as primary treatment is worth mentioning [4, 5]. Antioxidants, nutritional complements, prebiotics, probiotics and specifically defined nutritional schemes have been used in order to ameliorate the clinical course of patients with Crohn's disease and ulcerative colitis.

This review focuses on certain nutritional components that modulate the inflammatory response of the bowel. Certain nutritional elements play a significant role that can influence IBD. The aim of this review is to present a rational thesis regarding the use of nutritional agents in the management of IBD and to underline points that require further study in this very promising field of clinical nutrition (table 2).

## Oxidative Stress and Intestine

The release of free oxygen radicals (reactive oxygen species) is a significant factor of initiation and preservation of the inflammatory reaction in IBD. The gastrointestinal tract has the highest concentration in xanthine oxidase compared to any other tissue. Moreover, the high number of activated white cells that are normally located in the intestinal mucosa is multiplied in IBD, leading to the release of reactive oxygen species at high concentrations. In addition, the high quantity of catalase-negative bacteria at the distal part of the small intestine and the full length of the large intestine contributes to the release of large amounts of free oxygen radicals. For these reasons, the gastrointestinal tract has been described as a real 'time bomb' of free oxygen species which could be triggered in cases of IBD [6].

Low concentrations of active forms of oxygen may be beneficial or even necessary in certain processes like intracellular signaling and defense against microorganisms. However, higher concentrations play a role in the pathogenicity of some inflammatory diseases [7]. Some of the cytokines that promote the inflammatory reaction, which are overproduced in IBD, like interleukin 1β (IL-1β) and tumor necrosis factor-α (TNF-α), are involved in the release of free radicals.

Active oxygen species cause oxidative stress which results in cellular damage through chain reactions leading to breakage of macromolecules. The lipid peroxidation process is the result of chain reactions that are initiated from free radicals and in which a single free radical can cause the oxidation of a large number of cellular membrane molecules, like polyunsaturated fatty acids (PUFAs) [9]. The peroxidation of cellular membrane lipids may lead to functional alterations in the epithelial cells [9]. Active oxygen species can cause damage to the

**Table 3.** Mechanism of action of glutathione

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|---|
| – Antioxidant defensive action |
| – Modulating action in promoting intracellular processes |
| – Reduction of toxic activity of various electrophile elements |
| – Maintenance of the basic structure of protein thiols by preventing the oxidation of the sulfur group (–SH) or by reducing the formation of disulfide bonds that are promoted by oxidative stress |
| – Clearance of free oxygen radicals |
| – Preservation of cysteine reserve |
| – Modulation of DNA synthesis and immune function |

nucleic acid structure, endangering cellular survival and modifying gene expression, leading in that way to disrupted cellular activity. Thiol oxidation and carbonyl formation in proteins can rapidly aggravate cellular viability by inducing loss of function of enzymes, cellular receptors, cell signaling and signal transduction. Chemotactic subproducts of lipid peroxidation have a positive interaction in the acceleration and maintenance of the inflammatory process, which is a very important event in IBD pathogenesis (fig. 1). In addition, in order for the damages that were caused from oxidative stress in certain molecules to be restored, the defensive mechanism of the intestinal mucosa of the host has to clear the free oxygen radicals and the toxic forms of nitrogen dioxide that were produced in the intestine during the inflammatory reaction in order to prevent further extensive tissue damage. The host's defense includes antioxidative enzymes like intracellular superoxide dismutase and catalase, glutathione (GSH) hyperoxidase and reductase. These enzymes are complemented by antioxidants which include molecules derived solely from nutrition like vitamins C and E and GSH [10].

The potential of free oxygen species to influence the stimulus for the initiation of transcription and cytokine production directly correlates with comprehension of the pathogenesis of chronic IBD. A large amount of genes, enzymes and proteins as well as intracellular signaling elements and molecules are modulated from oxidative stress and cellular oxidation-reduction status [11]. The ability of antioxidants to modulate DNA damage and gene expression is an interesting research field of clinical nutrition. In experimental models of ischemia – reperfusion or necrotizing enterocolitis – the administration, before the ischemia, of antioxidants, deferoxamine, catalase and superoxide dismutase decreased the severity of the inflammatory bowel response [12].

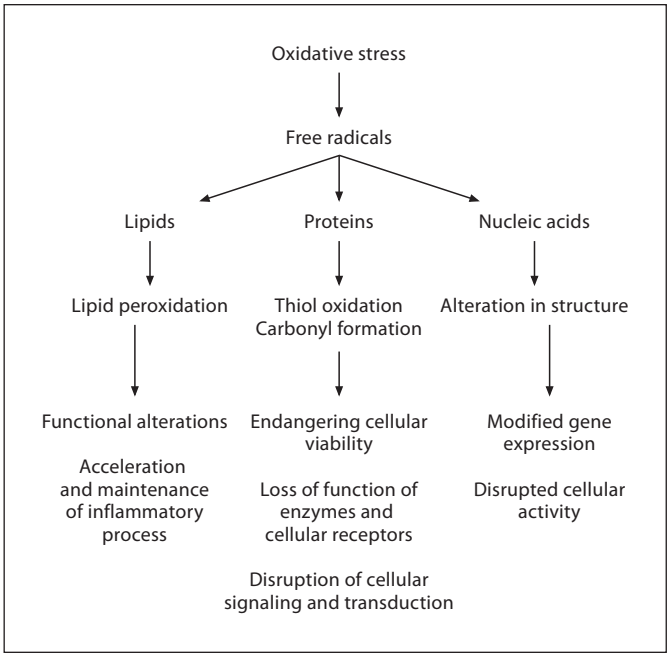


**Fig. 1.** Effect of oxidative stress on cellular function.

## Role of Nutritional Antioxidants in Inflammatory Bowel Disease

*Glutathione*

GSH is a significant intracellular peptide with multiple physiological functions, including an antioxidant defensive action as well as a modulating action in promoting intracellular processes [13, 14]. Also, GSH's vital functions include: (a) reduction of toxic activity of various electrophile elements, (b) maintenance of the basic structure of protein thiols by preventing the oxidation of the sulfur group (–SH) or by reducing formation of disulfide bonds that are promoted by oxidative stress, (c) clearance of free oxygen radicals, (d) preservation of cysteine reserve, and (e) modulation of DNA synthesis and immune function (table 3) [13]. Several studies have shown that oxidative stress promotes cellular apoptosis and that antioxidants, like those reduced from thiol GSH, may have a protective action [14].

During the progression of oxidative stress moderators, like superoxide and hydrogen peroxide, are formed which lead to further production of toxic oxygen radicals that can lead to lipid peroxidation with cell damage resulting as a consequence. Endogenic-produced hydrogen peroxide is reduced by GSH in the presence of selenium-dependent GSH peroxidase. Thus, GSH is oxidized to GSSG

which can then be reduced again to GSH by GSSG reductase, a reaction that is promoted by NADPH consumption, forming an oxidation-reduction cycle [15]. Despite the fact that hydrogen peroxide can be reduced by catalase, this enzyme is only present within the peroxisome. So, the presence of GSSG in the mitochondria is of vital significance in the defense against physiological and pathological oxidative stress. Consequently, sober oxidative stress can deplete cellular GSH [13]. The quotient of GSH/GSSG can be used to quantify tissue oxidative stress with the use of N-ethylmaleimide, which binds GSH in such a way that it cannot be oxidized to GSSG [16].

Several clinical conditions, including IBD, are related to reduced levels of GSH which result in a low cellular oxidation-reduction potential [17]. GSH and oxidation-reduction potential are components of the cell signaling system which can influence the translocation of the transcription factor and nuclear factor-κβ (NFκβ), which in fact regulate cytokines synthesis and molecule adhesion. Hence, a potential pathway to protect the cell from active oxygen species is restoration of the intracellular levels of GSH. This can be achieved by the administration of precursors of GSH, like glutamine or cysteine.

A crucial factor in GSH synthesis is the amount of cysteine in nutrition. Experimental studies have shown that inanition significantly reduces the levels of GSH, while recibation leads to the direct restoration of GSH repository to normal. The administration of L-cysteine prodrugs and GSH prodrugs in a murine model of DSS colitis has been shown to improve colonic lesions, normalize hepatic levels of GSH, regulate cytokines levels and in general attenuate the disease compared to control [18]. Also, the administration of *Lactobacillus fermentum*, a GSH-producing probiotic, in a TNBS model of rat colitis ameliorates colonic inflammation, reduces the levels of inflammatory mediators TNF-α and NO and increases GSH levels [19].

A limitation regarding interpretation of the antioxidant status in IBD is the measurement of antioxidant parameters in blood and not in intestinal tissue. Mucosal GSH in the large intestine in patients with Crohn's disease was found to be significantly lower both in the pathological and physiological parts of the bowel [20, 21]. It was also noted that dysthrepsia was directly related to the low levels of GSH. The authors concluded that low levels of tissue GSH in Crohn's disease could be responsible for the ongoing inflammatory reaction and should be a target of treatment with nutritional agents [21]. Also, in the inflamed large bowel mucosa of patients with IBD, it was found that vitamin C levels were significantly decreased, the activity of enzymes related with GSH synthesis was reduced [20], the activity of superoxide dismutase was decreased as were the levels of zinc and copper in addition with high levels of active oxygen species and lipid peroxidation [22]. Furthermore, in the serum of patients with active IBD, the levels of cysteine (basic precursor of GSH) were pathological [20]. So it becomes obvious that the decreased defensive potential of the GSH mechanism and of other antioxidants in patients with IBD is caused by both the low availability of nutritional agents and the inadequate synthetic potential of the organism.

*Vitamins A, C and E*

Vitamin E includes a group of tocopherols and tocotrienols, amongst which α-tocopherol has the highest biological action. Parts of vitamin E in lipoproteins and cellular membranes act like powerful antioxidants, protecting polyunsaturated fatty acids from the active free radicals [23]. Vitamin E is a drastic scavenger of free radicals which prevents the catastrophic chain reaction that characterizes the damage from free radicals. The action of vitamin E in the prevention of oxidative stress has been systematically studied and its positive results have been demonstrated in various conditions [17]. The balance between oxidants and antioxidants is a determinant factor of the cell immune function. Vitamin E, as an antioxidant, is considered to play a significant role in the maintenance of the integrity of the immune system. Also, it decreases the expression of adhesive molecules and affects the adhesion of white cells and the infiltration of tissues by white cells. Furthermore, vitamin E has a role in the metabolism of arachidonic acid, as it seems to increase the release of prostacyclins by increasing cellular phospholipase $A_2$ and cyclo-oxygenase. Ascorbic acid is an essential micronutrient for the physiological metabolic function as the human organism has lost the ability to synthesize vitamin C. Among its biological actions, vitamin C has a significant antioxidant role by scavenging active oxygen species [24]. Vitamin C also acts as a co-oxidant by recreating vitamin E and GSH from the α-tocopherol radical as part of the antioxidant grid [15, 25]. Vitamin A has antioxidant activity and regulates immune function as well as epithelial proliferation and differentiation (table 4) [26]. Vitamin A deficiency has been shown to induce colonic inflammation and amplify colitis, while vitamin A supplementation ameliorates colitis [26].

In a rat model of acetic acid colitis, the administration of vitamin E and selenium decreased macroscopic and mi-

croscopic damage of the colon and MPO activity compared to the control [27]. Patients with IBD have decreased levels of vitamins A, C and E [4, 28]. Rectal administration of D-alpha-tocopherol in patients with mild and moderately active ulcerative colitis has been shown to significantly decrease the average disease activity index and even cause remission due to the anti-inflammatory and antioxidative properties of vitamin E [29]. A recent study on severely ill patients showed that enrichment of the enteral nutrition solutions with vitamins A, C and E ameliorated the antioxidant defense [30]. In addition, in patients with inactive or mildly active Crohn's disease who have increased oxidative stress and lower antioxidant vitamins levels, supplementation with vitamins C and E significantly reduced the oxidative stress compared to control [31].

On the other hand, the interaction of vitamin C with free, catalytic active metal ions could participate in oxidative damage by releasing hydroxyl and alkoxyl radicals [32]. Low levels of ascorbic acid may show extensive consumption of it as an antioxidant or may be due to the extremely inadequate uptake of vitamin C or may be because ascorbic acid is consumed in order to recreate GSH and vitamin E [15]. It should be noted that because of the pro-oxidative potential of ascorbic acid particular attention should be paid when trying to restore its levels to normal [33]. This is further highlighted in patients receiving ferrous as a supplement as ascorbic ferrous negatively affects the function of intestinal cells [34].

*Free Metals Related to Antioxidant Mechanisms: Selenium, Copper, Zinc*

Three primary antioxidant enzymes have been described in mammals: superoxide dismutase, catalase and GSH peroxidase [35]. Superoxide dismutase utilizes the metals copper-zinc (Cu-Zn) or manganese (Mn) as coenzymes for the conversion of the superoxide radical to hydrogen superoxide, which then is converted to water from catalase and GSH peroxidase [15]. Selenium is a basic micronutrient and an essential component of the catalytic part of GSH peroxidase. Selenium deficiency is directly related with complete decrease of GSH peroxidase activity in several tissues, which leads to an increase of oxidative stress. In conditions related with oxidative stress and inflammatory reactions, high selenium efficiency has been shown to have a positive effect [36]. The supplemental administration of selenium has been shown to increase the GSH levels in patients that have undergone kidney transplantation [20].

In vitro studies suggest that antioxidants can repress the production of cytokines from blood mononuclear cells in patients with IBD [37]. However, results from intestinal mucosa biopsies revealed that the production of cytokines had only been slightly affected. Furthermore, in a TNBS rat model of colitis, a high-selenium diet significantly increased selenium content in the colonic tissue, preserved the tissue architecture, attenuated neutrophil infiltration and decreased tissue MPO activity [38]. Also, in a mice model of DSS colitis, Zn administration significantly decreased the disease activity index compared to control [39].

Decreased levels of selenium are more commonly found in patients with extensive enterectomy of the small intestine, but can also be found in patients with IBD [40, 41]. Also, lower levels of zinc may also be noted [42]. Smoking and other environmental factors can also affect the antioxidant status. Various explanations can be proposed regarding the low levels of vitamins. Low antioxidant status has been related to disease activity [43]. In a phase II study, the use of bovine Cu-Zn-superoxide dismutase and deferoxamine decreased the severity of IBD [44]. The free radical scavengers allopurinol and dimethyl sulfoxide proved to have a positive effect in the treatment of ulcerative colitis [45]. A recent double-blind study in patients with inactive Crohn's disease revealed that the administration of antioxidant supplements had increased the levels of selenium and vitamins C and E in serum as well as the activity of superoxidase dismutase [46].

Seidman et al. [15] have noticed that in various clinical studies regarding the concentration of antioxidants, vitamins and metals in patients with IBD, the levels of antioxidants were decreased during the active phase of IBD, especially in dysthreptic patients with Crohn's disease. Based on previous studies and the fact that the levels of trace elements are abnormal in patients with IBD, which

**Table 4.** Mechanism of action of vitamins

| | |
|---|---|
| – | Vitamin E |
| | Antioxidant |
| | Scavenger of free radicals |
| | Reduces protein kinase C |
| | Decreases the expression of adhesive molecules |
| | Increases prostacyclin release |
| – | Vitamin C |
| | Scavenger of free radicals |
| | Recreation of vitamin E and glutathione |
| – | Vitamin A |
| | Antioxidant |
| | Regulates immune function and epithelial proliferation |

**Table 5.** Mechanism of action of n–3 polyunsaturated fatty acids

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| – Decrease the production of inflammatory cytokines ($LTB_4$ and thromboxane $A_2$) |
| – Suppress the activity of leukocytes and natural killer cells |
| – Limit antibody synthesis |
| – Increase the production of less inflammatory cytokines ($LTB_5$) |
| – Act as free radical scavengers |
| – Repress platelet coagulation |

leads to reduced free radical scavenging action and continued inflammatory processes, supplementation of trace elements in patients with IBD is further supported [47].

### Role of Nutritional Lipids in the Treatment of Inflammatory Bowel Diseases

A prevalent hypothesis that supports the use of fish oil in the treatment of autoimmune diseases is that 'western diets' (high n–6, low n–3, quotient 25:1) are consumed in countries with a high prevalence of autoimmune diseases, while in growing countries the percentage is much lower (the n–6/n–3 quotient is 2:1). Polyunsaturated fatty acids (PUFAs) belonging in the n–3 subcategory are considered to have a beneficial effect in the management of IBD by repressing cytokine production and modulating the production of eicosanoids. n–3 PUFAs are bound from immune cells instead of n–6 fatty acids and so production of inflammatory eicosanoids like leukotriene $B_4$ ($LTB_4$) and thromboxane $A_2$ is decreased and inflammation is limited [48, 49]. Also, they cause a shift from leukotriene LTB4 production to LTB5 production which is less bioactive. In addition, n–3 PUFAs repress the activity of leukocytes, proliferation and synthesis of inflammatory cytokines (TNF, IL-1, etc.), activity of natural killer cells, synthesis of antibodies as well as expression of the surface membrane molecule of macrophages [48]. Except for the alteration in eicosanoid production, leukocyte activity and cytokine synthesis, it is proposed that n–3 fatty acids contribute to the modulation of the inflammatory process and can act even as scavengers of free radicals [15]. In addition, they repress platelet coagulation, a very significant process in the hypothesis that the pathogenesis of Crohn's disease includes multifocal intestinal infarcts (table 5). In general, nutritional lipids which are rich in n–6 PUFAs increase the cytokine response in contrast to the lipids rich in n–3 PUFAs which have the exact opposite action.

The mechanism through which the PUFAs perform their immunomodulating action still remains unclear. Several hypotheses have been proposed including an alteration in the membrane phospholipids, alteration in the eicosanoids production, formation of lipid peroxisomes as well as regulation of gene expression. Sensitivity in the anti-inflammatory action of fish oils is regulated from a genotypic diversity in the genes influenced by lymphotoxin-α [50]. Both the quantity and the quality of nutritional lipids configure bowel immune function. So, for example, the reaction of peritoneal macrophages of rats regarding the production of IL-1 and IL-6 after the administration of TNF-α is significantly influenced by the nutritional administration of linoleic acid in a percentage representing 1–4% of the administered energy. The equivalent percentage in humans has not been determined. The uptake of long-chain fatty acids stimulates lymphocyte blastogenesis as well as the fluidity of intestinal lymphatic vessels. Also, it increases the migration of T lymphocytes to Peyer's patches, possibly by increasing the adhesion molecules like $\alpha_4$-integrin and L-selectin. Lipoproteins can stimulate lymphocyte activity by mechanisms dependent or not by receptors [51]. Changes in the nutritional uptake of lipids cause alterations in the regulation mechanisms of physiological uptake of fatty acids from the intestinal cells.

Experimental studies have shown the positive effect of eicosapentaenoic acid in the treatment of cachexy and in the progress of the inflammatory process [52, 53]. The administration of n–3 fatty acids has been studied in various inflammatory and autoimmune diseases and seems to contribute to abatement of activity of these diseases and decrease in the usage of anti-inflammatory drugs [54, 55]. However, in an in vitro study using blood mononuclear cells of individuals receiving supplemental fish oil, the production of TNF-α after the administration of lipopolysaccharide was only repressed in half of them [50].

One of the theoretical benefits of the certain types of diet in the treatment of IBD may arise from a change in the type or quantity of nutritional lipids. A proposed hypothesis suggests that the efficiency of enteral nutrition as a primary treatment for active Crohn's disease depends on the concentration of PUFAs which act as the primary substances for the synthesis of eicosanoids through the arachidonic acid cycle [2]. It has been proved that enteral nutrition, as the primary treatment for Crohn's disease, decreases the production of Th-1 lymphokine (IL-2, IFN-γ) in a likewise fashion as cyclosporine [56]. A possible explanation is alteration in the uptake of nutritional lipids. Intravenous administration of eicosapentaenoic acid

in patients with active Crohn's disease affected leukotriene production (LTs) by increasing the leukocyte levels of $LTB_5$ [57]. Cholecystokinin is considered to increase the monovalent intracellular calcium content in large monocytes and promote mitogenesis of the lymphocytes [58]. Hence, it can be hypothesized that diets that increase cholecystokinin secretion ameliorate the immune response of the bowel, while, on the other hand, nutritional modulations that decrease cholecystocine secretion may enhance the production of inflammatory mediators [59, 60]. Epidemiological studies in Japan have shown that the recent increase in the incidence of Crohn's disease is strongly related to the increased uptake of n–6 PUFAs, zoic milk protein and the quotient of n–6/n–3 consumption [61]. Administration of nutritional supplements containing n–3 fatty acids significantly decreased the relative proportion of arachidonic acid while it increased the levels of eicosapentaenoic and docosahexaenoic acid of serum phospholipids and adipose tissue in patients with inactive Crohn's disease [46]. The combination of n–3 fatty acids and antioxidants would favor the production of eicosanoids with parallel abalienation of the mechanism which promotes the inflammatory reaction [46].

n–3 fatty acids could theoretically be efficient in the treatment of active ulcerative colitis, hence decreasing the need for steroids as well as preventing the recurrence of ulcerative colitis and of Crohn's disease [62]. Supplemental administration of nutritional fish oils causes remission of symptoms in ulcerative colitis and decreases $LTB_4$ concentration in the rectum [15]. In a significant study, Belluzzi et al. [63] noticed a significant decrease in the recurrence rate of patients with Crohn's disease who were randomly taking n–3 fatty acids supplements. A group of 78 patients with Crohn's disease with high possibility of recurrence were randomized to receive daily 2.7 g of an enteric-coated fish oil supplement (2/3 eicosapentaenoic acid, 1/3 docosahexaenoic acid) or placebo. After 1 year of treatment, 59% of the patients in the fish oil group remained in remission compared with only 26% in the placebo group, with the difference being statistically significant [63]. Monovariant statistical analysis demonstrated that the fish oil was exclusively responsible for the decrease of recurrence possibility compared with the placebo group (OR 4.2, 95% CI 1.6–10.7). However, other studies regarding n–3 fatty acids did not confirm these findings [62, 64]. The discordance regarding these studies may be related with the effect of other simultaneous treatments, type and dose of n–3 fatty acids supplements, selection mode of patients, type of basic diet, and treatment compliance. Based on data from an open pilot study, Tsujikawa et al. [65] support that a diet rich in n–3 fatty acids (n–3/n–6 = 0.5) based on rice, boiled fish and soup can be beneficial in the maintenance of disease remission, without the need of supplemental fish-oil administration. However, it is difficult for these types of diets to be applied in North America and Europe where n–6 consumption is very high. The use of an 'n–3 PUFA food exchange table' can prove beneficial for patients with IBD as it alters the fatty acid composition of the cell membrane by increasing the n–3/n–6 ratio and influences clinical activity in patients with IBD [66]. However, in two large double-blind, placebo-controlled, randomized studies investigating if the oral administration of N3PUFAS is more effective than placebo in preventing the relapse of Crohn's disease, it was proved that treatment with N3PUFAs was not more effective than placebo in maintaining remission in Crohn's disease [67]. So it is obvious that further studies are necessitated in this area of clinical nutrition.

### Short-Chain Fatty Acids and Inflammatory Bowel Diseases

Short-chain fatty acids are produced in the lumen of the large intestine from anaerobic fermentation of hydrocarbons and proteins that were not absorbed in the small intestine. Short-chain fatty acids and, especially, butyrate play a very important role in the biology of the large bowel epithelium, representing the primary energy source of the large bowel cell. Deficiency of short-chain fatty acids in the intestinal lumen is related with epithelium atrophy and inflammation, as for example in the diversion colitis. In ulcerative colitis, the reduced oxidation of butyrate from the large bowel cells was implicated for the pathogenesis of the disease, suggesting that the disease was a consequence of the metabolic insufficiency of the epithelial cells. Butyrate enemas are very promising in the treatment of ulcerative colitis, especially when the disease is located in the distal large bowel [68]. It would be interesting if there was a 'carrier' that could release butyrate in the proximal colon rather than energy production being dependent from the formation of nonabsorbable hydrocarbons. Butyrate is a potential promoter of the proliferation and differentiation of large intestine epithelial cells and reduces the paracellular permeability, possibly due to the promotion of intestinal cell differentiation. Butyrate also promotes the action of GSH transferase in the intestinal epithelial cells. Short-chain fatty acids may also be useful in other inflammatory conditions of the bowel.

The histopathological study of the small and large intestine after the administration of cytotoxic drug Ara-c in mice revealed less damage in mice receiving short-chain fatty acids [69]. This therapy represents a possible method to decrease inflammation and to reduce mucositis caused by chemotherapy. Also, the addition of butyrate to standard mesalazine treatment in patients with ulcerative colitis has led to marked improvement of symptoms and endoscopic appearance of the mucosa, thus proving effective in reducing disease activity [70]. In addition, in an open-label randomized clinical trial in patients with ulcerative colitis, the administration of *Plantago ovata* seeds, colonic fermentation of which yields butyrate, was proved to be as effective as mesalamine in maintaining remission in ulcerative colitis for 12 months [71]. Moreover, acetate and propionate may also be useful in the treatment of IBD as they can cause suppression of NF-κB reporter activity, immune-related gene expression and cytokine release in vitro [72].

### Modulation of Mucosal Immunity from Amino Acids

Among the several hypotheses that have been proposed for the interpretation of the benefits resulting from the use of oligopeptide solutions is the one suggesting that the benefit is a result of the sequestration of intraluminal proteinic antigens, which could stimulate the immune response of the bowel. Another hypothesis suggests that certain peptides have immunomodulating ability. For example, β-casein and its opioid peptides, known as β-casomorphins, have immunomodulating actions like the promotion of antibody synthesis and phagocytic activity. Bovine β-casein enhances the production of superoxides from the neutrophils as well as the proliferation of T and B lymphocytes. Also, it increases IL-11 production from macrophages. These results suggest that β-casein shows modulating actions, mostly immunostimulating, both in the innate and the acquired immune response. So the sequestration of this nutritional agent from the diet would theoretically affect the bowel immune response [73].

Another mechanism that underlines the beneficial action of certain types of diets in Crohn's disease is the ability of glutamine to stimulate the integrity of the intestinal mucosal barrier and promote its restoration during the inflammatory reaction [74]. Certain roles have been attributed to glutamine as an immunomodulating agent as the supply of the preferred energy source for the intestinal epithelial cells, the protection of the function of the intestinal mucosal barrier, and the fact that it is a basic nutritional element for the cell immune function [50]. Bowel dysfunction is related to increased bacterial translocation and bacterial product translocation, like endotoxins, or even with the endangering actions of cytokines which promote the inflammatory reaction, such as TNF-α. Parenteral nutrition, without glutamine, has deleterious effects in the defense ability of the immune mechanism of the intestinal mucosa which are manifested with reduced production and secretion of IgA, IL-4 and IL-10 and reduction of mRNA expression from cells of the intestinal basal membrane. The supplemental administration of glutamine protects and preserves the action of these intestinal-protecting immune mechanisms within normal levels. The nutritional administration of glutamine in rats with trinitrobenzene sulphonic acid-induced colitis restored the production of cytokines which promote the inflammatory reaction (IL-8 and TNF-α) [75]. Furthermore, glutamine is a substrate for the synthesis of GSH [50] helping in the protection of intestinal mucosa from oxidative stress, and also glutamine supplementation can improve colonic barrier function [76]. Also, the combined administration of glutamine and arginine decreased proinflammatory cytokines release from active colonic biopsies of patients with Crohn's disease [77].

The beneficial effects of more amino acids have been studied recently. In a porcine model of DSS colitis, supplementation of L-tryptophan not only ameliorated clinical symptoms and increased weight gain but also improved histological scores and decreased the expression of proinflammatory cytokines [78]. In addition, in a rat model of DSS colitis the administration of L-threonine, L-proline, L-cysteine and L-serine improves colonic protection and mucosal healing by an increase in mucin synthesis and by promoting gut microflora reequilibration [79].

The production of acute-phase proteins and GSH is significantly affected by the uptake of proteins and especially of sulfur and sulfur-related amino acids. The role of sulfur amino acids and polyamines as immunonutritional factors was recently reviewed [80]. The levels of dietary cysteine and methionine directly affect the production of GSH.

### Prebiotics, Probiotics and Bowel Immune Response

Prebiotics and probiotics represent a very promising treatment for certain infections and inflammatory and atopic bowel disorders. Intestinal microflora play a significant role in the promotion and maintenance of chron-

ic bowel inflammation. Prebiotics are nondigestible food ingredients that by elective stimulation of the growth and/or activity of one bacterium or a group of bacteria in the large bowel have a beneficial role on the health of the host [81]. The supplementation of prebiotics contributes to growth of the intestinal mucosa, maintenance of the intestinal mucosal barrier, balance of body fluids and electrolytes, supplementation of the body with energy and nutritional agents, as well as enhancement of the defense against pathogenic micro-organisms, modulation of the intestinal mucosa immune response and stimulation of antibody-mediated immunity (table 6).

The two well-known prebiotics are inulin and oligofructose, which are vegetal carbohydrates and are called fructans. Inulin has a larger chain length than oligofructose and is thus less soluble. Another probiotic, pectin, is considered to act as an antioxidant and promote restoration of the intestinal microbiota [82]. The daily intake of prebiotics in Western societies varies from 3 to 13 g per day [83].

The administration of green banana (250 g/l of food) or pectin (2 g/l of food) has been tested in children with severe persisting diarrhea who were fed almost exclusively on rice. Prebiotics administration resulted in reduced frequency and duration of diarrhea, frequency of vomiting and decreased need of fluid for the proper hydration of patients even from the third day of treatment in 59% of patients fed with green banana and 55% of patients fed with pectin compared to 15% of patients fed on rice [84]. Prebiotics have been mostly used in models of experimental colitis, but the administration of fructo-oligosaccharide for 21 days in patients with Crohn's disease significantly decreased disease activity [85], while the administration of inulin in patients with pouchitis has resulted in decrease in disease activity index and in histological and endoscopy scores [86].

Probiotics are live microorganisms contained in food, that can alter the host's intestinal microflora and hence can have beneficial effects to the host's health. Priobotics are human in origin, nonpathogenic, resistant to gastric secretions and bile salts, have the ability to adhere to and remain on the gastrointestinal epithelium and produce antibacterial agents and modulate the intestinal mucosa immune response [15]. The most common and widely used probiotics are strains of *Lactobacillus* and *Bifidobacterium* [87]. Typical examples of probiotics are *Lactobacillus rhamnosus* GG, *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus acidophilus* [88]. Regarding their mechanism of action, experimental models show that probiotics prevent the adherence and translocation of pathogenic bacteria to the intestinal mucosa and restore nonphysiological intestinal permeability. They also stimulate the protective immune response of the intestinal mucosa by increasing sIgA and IL-10 and decreasing TNF-α and IFN-γ [15]. Administration of probiotics prevented the colitis in IL-10-deficient mice [89]. In addition, they have antimicrobial activity by reducing the intraluminal pH and by producing short-chain fatty acids and $H_2O_2$. Finally, they enhance the integrity of the mucosal barrier by preventing mucosal apoptosis and stimulating expression of the MUC gene (table 7) [15]. Supplementation of the synthetic probiotic mixture, VSL#3 (including *Streptococcus thermophilus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei* and *Lactobacillus bulgaricus*) seems to restore colonic mucosa barrier function in experimental colitis [83]. For example, *Lactobacillus* GG has been successfully used for the prevention and treatment of recurrent colitis from *Clostridium difficile*.

While a lot of nonantibiotic drugs modulate the immune response in IBD, probiotics have the ability to alter the intestinal microflora. Recent studies about the effect of VSL#3 in the treatment of pouchitis [90] and ulcerative colitis [91] had promising results. Moreover, in a pilot study of patients with acute distal ulcerative colitis, the administration of Synbiotic 2000 enema twice a day for 2 weeks significantly decreased episodes of diarrhea, visible blood in stool, nightly diarrhea and urgency and im-

**Table 6.** Mechanism of action of prebiotics

- Growth of the intestinal mucosa
- Maintenance of the intestinal mucosal barrier
- Balance of body fluids and electrolytes
- Supplementation of the body with energy and nutritional agents
- Defense against pathogenic microorganisms
- Modulation of the intestinal mucosa immune response
- Stimulation of the antibody-mediated immunity

**Table 7.** Mechanism of action of probiotics

- Produce antibacterial agents
- Modulate the intestinal mucosa immune response
- Prevent the adherence and translocation of pathogenic bacteria
- Restore the nonphysiological intestinal permeability

proved the consistency of the stool [92]. However, due to the variability of the strains and the heterogeneity of the patients, it could not be assumed that similar beneficial results can be expected for patients with Crohn's disease, but taking the current understanding for the pathogenesis of the disease into consideration, the alteration of intestinal microflora is a very possible pathway of action for the biotherapeutic agents in the treatment of Crohn's disease. There are a few data regarding the administration of probiotics in patients with Crohn's disease. In a double-controlled study of patients with Crohn's disease in remission, the patients received either 5-ASA (3 g/day) or 5-ASA in combination with *Saccharomyces boulardii* (1 g/day) [93]. After 6 months, the recurrence was 38% (6/16) in the 5-ASA group but only 7% (1/16) in the 5-ASA and probiotic group. Also, probiotics have proved useful in the treatment of ulcerative colitis. The oral administration of probiotic *Escherichia coli* Nissle 1917 has been proved to be as effective as mesalazine in preventing relapse in patients with ulcerative colitis [94]. Furthermore, another probiotic, *Lactobacillus* GG, was shown to be effective and safe for maintaining remission in patients with ulcerative colitis [95].

### N-Acetylglucosamine

The catabolism of glycosaminoglycan is a significant consequence of the inflammatory process on the mucosal surface. Inhibition of metalloproteinase action can be beneficial in the treatment of chronic inflammation. In a pilot study, Salvatore et al. [96] tested the nutritional agent N-acetylglucosamine (GlcNAc), an aminosaccharide, which accumulates in glycosaminoglycans and glycoproteins, forming a substrate for tissue repair mechanisms, by administrating 3–6 g GlcNAc daily to pediatric patients with Crohn's disease. Among the 12 patients that received GlcNAc, 8 had significant improvement while 4 had to undergo enterectomy. Also, of the 7 patients with symptomatic stenosis caused by Crohn's disease, 3 had to undergo surgical treatment after a mean period of 2.5 years, while in the others there was significant endoscopic or radiographic improvement. In addition, there was histologic improvement as well as a significant increase in glycosaminoglycan concentration in the intestinal epithelium and basal membrane and an increase of intracellular GlcNAc. This pilot study provides preliminary results that support this new therapeutic approach with the use of nutritional agents.

### Dietary Particles

Nonphysiological intestinal permeability is directly related to the activity of IBD. The possible immunologic effectiveness of refined particles which are present in the nutrition was the objective of recent studies. A typical Western diet contains more than $10^{12}$ refined particles consumed daily. Using an experimental model of intestinal culture, a study showed that the in vitro exposure of intestinal biopsies to titanium dioxide particles conjugated with liposaccharides significantly increased IL-1 production [17]. This study supports the hypothesis that dietary particles are not immunologically inactive and that they play a significant role in the increase of the physiological intestinal cell activity against endogenic intraluminal bacterial antigens, while other studies show that despite the fact that dietary microparticles alone have limited effects on basic macrophage functions, they have the ability to act as adjuvants and could aggravate the ongoing inflammatory response [97]. So, it could be assumed that certain types of diets may be beneficial if they contain reduced quantities of refined particles. Microparticles accumulate in the phagocytes of the lymphadenoid tissue of the bowel. In a recent double-blinded study, it was observed that patients with active Crohn's disease who received diets containing a low concentration of microparticles in combination with corticosteroids had a higher possibility of achieving disease remission [98]. This fact represents another possible benefit of this certain type of diet. However, there are studies that could not find any evidence that reducing microparticle intake aids remission in active Crohn's disease [99].

### Conclusion

IBDs are considered as under-research diseases because of the difficulty of finding a specific causative factor and because of the lack of aimed treatment. Immunomodulating agents and corticosteroids have been used for the treatment of IBD without always having the desired outcome. Nutritional agents have been used and researched for the treatment of these diseases, including antioxidants (GSH, vitamins A, C and E, selenium and zinc), lipids (n–3 polyunsaturated fatty acids and short chain fatty acids), amino acids, prebiotics and probiotics, GlcNAc, and dietary particles. The possible mechanisms of action of the nutritional therapeutic treatment for the control of the activity of the IBD are: (a) sequestration of intraluminal antigens, (b) modulation of the immune re-

sponse of the bowel, (c) amelioration of the antioxidant status, (d) alteration in the uptake of polyunsaturated fatty acids (n–6/n–3 fatty acids), (e) enhancement of the restoration/function of the intestinal mucosal barrier, (f) regulation of the intestinal microflora, (g) regulation of the intestinal motility, (h) regulation of the bile-pancreatic secretions, and (i) sequestration of nutritional particles. However, results have not always being satisfactory, but in many cases they were promising. So, more studies, especially double-blind control studies, are still needed in order to clarify the role of nutrition in the modulation of IBD and its management.

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